Application Serial No. 10/554,288 Amendment After Final dated 5 March 2008

Reply to Office Action dated 24 January 2008

AMENDMENTS TO THE CLAIMS:

This following listing of claims will replace all prior versions, and listings of claims in

the application.

Listing of Claims:

Claim 1 (Currently Amended): A method of isolating a  $\beta$  (1-3)  $\beta$  (1-4) glucan from a

milled cereal grain or a milled part of the cereal grain, comprising:

(i) extracting the milled cereal grain or the milled part of the cereal grain with an

alkaline solution having a value of pH of between 9 to 10 for a period of time of about 15 to

about 45 minutes to produce an extract containing at least about 0.4 weight percent  $\beta$  (1-3)  $\beta$  (1-

4) glucan;

(ii) removing insoluble material, and removing particulate material having a particle

size of greater than about 0.2  $\mu$ m from said extract to produce a purified extract comprising  $\beta$  (1-3)  $\beta$  (1-4) glucan having a particle size of less than 0.2  $\mu$ m, wherein the step of removing

particulate material comprises:

using microfiltration to filter out material having a particle size of greater than about 0.2

μm from said extract by microfiltration and produce a filtrate comprising β (1-3) β (1-4) glucan

having a particle size of less than 0.2 μm;

(iii) adding from between 10% to 20% (vol/vol) of a C<sub>1</sub>-C<sub>4</sub> alcohol to the purified

extract to precipitate the  $\beta$  (1-3)  $\beta$  (1-4) glucan, and

(iv) isolating the  $\beta(1-3) \beta(1-4)$  glucan.

Claim 2 (Previously Presented): The method of claim 1, wherein the C<sub>1</sub>-C<sub>4</sub> alcohol is

selected from the group consisting of methanol, ethanol and isopropanol.

Page 2 of 9

Application Serial No. 10/554,288 Amendment After Final dated 5 March 2008 Reply to Office Action dated 24 January 2008

Claim 3 (Previously Presented): The method of claim 2, wherein the C<sub>1</sub>-C<sub>4</sub> alcohol is ethanol

Claim 4 (Currently Amended): The method of claim 1, wherein, said step of removing particulate material <u>further</u> comprises the <u>following steps prior to the microfiltration step</u>:

one, or more than one step of adding a flocculant, a coagulant or both a flocculant and a coagulant to said extract to coagulate particulate material having a particle size of greater than about 0.2 um, and removing coagulated material from said extract; and

digesting starch material in said extract, and

filtering out particulate material having a particle size of greater than about 0.2 µm from said extract to produce a purified extract.

Claim 5 (Original): The method of claim 4, wherein, in said step of digesting, said starch material is digested with an enzyme.

Claim 6 (Original): The method of claim 5, wherein prior to digesting said starch material, said alkaline solution is neutralized.

Claim 7 (Original): The method of claim 6, wherein following the digestion of said starch material, said enzyme is inactivated.

Claim 8 (Original): The method of claim 7, wherein said enzyme is inactivated by acidifying the neutralized solution.

Claim 9 (Original): The method of claim 5, wherein said enzyme is an amylase.

Claim 10 (Original): The method of claim 9, wherein said amylase does not require a calcium cofactor.

Application Serial No. 10/554,288 Amendment After Final dated 5 March 2008

Reply to Office Action dated 24 January 2008

Claim 11 (Original): The method of claim 1, wherein the cereal is selected from the

group consisting of a cultivar of barley, a cultivar of oat, a cultivar of wheat, a cultivar of rye, a

cultivar of sorghum, a cultivar of millet, and a cultivar of corn.

Claims 12-13 (Canceled).

Claim 14 (Original): The method of claim 1, wherein said step of adding (step iii) is

conducted at a temperature of from about 1°C to about 10°C.

Claim 15 (Previously Presented): The method of claim 1, further comprising one, or

more than one step of dissolving the isolated  $\beta$  (1-3)  $\beta$  (1-4) glucan in an aqueous solution,

precipitating the β (1-3) β (1-4) glucan by adding between 10% to 20% (vol/vol) of the C<sub>1</sub>-C<sub>4</sub>

alcohol to the aqueous solution, and isolating the  $\beta$  (1-3)  $\beta$  (1-4) glucan.

Claim 16 (Currently Amended): A method of isolating a  $\beta$  (1-3)  $\beta$  (1-4) glucan from a

milled cereal grain or a milled part of the cereal grain, comprising:

(i) extracting the milled cereal grain or the milled part of the cereal grain with an

alkaline solution having a value of pH of between 9 to 10 about 9.25 to about 9.75 for a period of

time of about 15 to about 45 minutes to produce an extract comprising at least about 0.4 weight

percent  $\beta$  (1-3)  $\beta$  (1-4) glucan;

(ii) removing insoluble material, and removing particulate material having a particle

size of greater than about 0.2  $\mu m$  from said extract to produce a purified extract comprising  $\beta$  (1-

 $\underline{3)\,\beta\,(1\text{--}4)\,\text{glucan having a particle size of less than 0.2 }\mu\text{m}$ , wherein the step of removing

particulate material comprises:

one, or more than one step of adding a flocculant, selected from the group consisting of a

polyaerylamide, a quaternary aerylate salt and a natural flocculant macromolecule, a coagulant,

selected from the group consisting of alum, lime, ferric chloride, ferrous sulfate, an organic

Page 4 of 9

Application Serial No. 10/554,288

Amendment After Final dated 5 March 2008

Reply to Office Action dated 24 January 2008

polymer and a synthetic polyelectrolyte with anionic or cationic functional groups, or both the flocculant and the coagulant to said extract to coagulate particulate material having a particle size

of greater than about 0.2 µm, and removing coagulated material from said extract;

enzymatically digesting starch material in said extract, and

using microfiltration to filter filtering out particulate material having a particle size of greater than about 0.2 um from said extract to and produce the purified extract comprising \( \beta \) (1-

3) β (1-4) glucan having a particle size of less than 0.2 μm as a filtrate;

adding about 10% to about 25% (vol/vol) of a C1-C4 alcohol to the purified

extract to precipitate the B(1-3) B(1-4) glucan, and

isolating the  $\beta(1-3)$   $\beta(1-4)$  glucan. (iv)

Claims 17-27 (Canceled).

Claim 28 (Previously Presented): The method of claim 4, wherein the flocculant is

selected from the group consisting of a polyacrylamide, a quaternary acrylate salt and a natural

flocculant macromolecule, and the coagulant is selected from the group consisting of alum, lime, ferric chloride, ferrous sulfate, an organic polymer and a synthetic polyelectrolyte with anionic

or cationic functional groups.

Claim 29 (Previously Presented): The method of claim 1, wherein about 15% to about

17% (vol/vol) of the C<sub>1</sub>-C<sub>4</sub> alcohol is added to the purified extract in step (iii).

Claim 30 (Previously Presented): The method of claim 16, wherein about 10% to about

20% (vol/vol) of the C1-C4 alcohol is added to the purified extract in step (iii).

Claim 31 (Previously Presented): The method of claim 16, wherein about 15% to about

17% (vol/vol) of the C<sub>1</sub>-C<sub>4</sub> alcohol is added to the purified extract in step (iii).

Page 5 of 9